aligned with the MT axis, indicating the existence of a shorter time-scale transition period on the MT before free diffusion begins. We also correlated the changes in early endosome motion with the position of other organelles and the cytoskeleton. Pauses in directed movement spatially correlate with regions of dense MTs as well as other early endosomes and the endoplasmic reticulum, suggesting that early endosomes interact with these cellular features during their transport.
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3348-Pos Board B503
Time-Lapse Super-Resolution Imaging of Apical Membrane Protein Domains in Live Filamentous Fungi
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The core mechanism of the asymmetric and the polarized cell growth is a conserved process among many organisms. This process involves complex concerted interplay of dynamic rearrangements of cytoskeletons, the mobilization of proteins from intracellular pools, active transport of vesicles to fusion sites and the accumulation of ''cell end marker'' proteins. While many of the key components for the polarized growth are known, the detailed mechanism of these complex processes is still unclear. TeaR is one of the cell end marker protein that plays a crucial role in the initiation and the maintenance of straight-growth in a filamentous fungi, *Aspergillus nidulans*. While many end marker proteins show a single accumulation of the domain near the end of the cell, widefield fluorescence microscopy data shows a distribution of multiple TeaR domains. Our current colocalization studies strongly suggest that majority of TeaR domains consist of accumulated secretory vesicles that are docked near the plasma membrane. This suggests that these domains mark the exocytosis sites of the hypha. In order to elucidate the detailed architecture of TeaR domains near the hypha tip, we have performed a super-resolution microscopy imaging, photoactivated localization microscopy (PALM) in live filamentous fungi. PALM imaging of TeaR fused with a photoconvertible fluorescent protein, mEosFP*thermo*, shows a cluster size distribution centered around 140 nm. Time-lapse PALM imaging further reveals that these clusters in growing cells are highly dynamic. Processes resemble vesicle trafficking along the cytoskeleton, docking, accumulation of vesicles, dispersion of proteins along the membrane and the growth of the membrane has been observed. We present the affect of key deletion mutants that influence the directionality of the hypha growth on these dynamics.

3349-Pos Board B504
Magnetic Manipulation of Axonal Transport in Live Neurons
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Retrograde neurotrophic signals, from the axon terminal to the cell body, are essential for the survival and function of neurons. Axonal microtubules serve as polarized tracks for molecular motor proteins driving the signaling endosomes from the axon terminal to the cell body. The robustness of this long-distance transport and the direction specificity can be attributed to the cooperative mechanics of multiple motors and/or specific coordinators in vivo. Noninvasive external force control of axonal endosomes in live neurons is a challenging prospect, which can unravel the transport machinery and the direction regulation mechanisms in vivo. Here, we present an integrated methodology based on microfluidic neuron culture, high-gradient magnetic trapping and pseudo-TIRF imaging that permits external control of axonal endosome transport in live neurons via magnetic forces. We fabricated a novel microfluidic device for neuron culture by patterned electrodeposition of soft micromagnets on glass coverslips. In the presence of an external magnetizing field, the soft micromagnetic pattern gives rise to local zones of high magnetic gradients. By culturing neurons in this device, with axons aligned along these high gradient zones, we can exert pN forces on axonal endosomes carrying magnetic nanoparticles ($<$100 nm). The magnetic forces can be designed to either assist/oppose the molecular motor forces driving the axonal endosomes. We have successfully compartmentalized DRG neurons in prototype magnetic devices. Further, high-resolution tracking of axonal endosomes under external load and stochastic modeling reveal that A) motors of both polarity are involved even in the apparent unidirectional transport of axonal endosomes and B) mechanical force balance is a critical component in determining the endosome directionality.

3350-Pos Board B505
Kinesins in Caenorhabditis Elegans Chemosensory Cilia Relay to Drive Intracellular Transport
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Cilia are organelles emanating from the surface of nearly every cell in the body playing crucial roles in cell signaling and motility. For cilium development and maintenance, intraflagellar transport (IFT) along the ciliary axoneme is essential. In Caenorhabditis elegans chemosensory cilia, IFT is driven by two kinesin-2's - kinesin-II and OSM-3 - carrying cargo toward the tip of the cilium and a cytoplasmic dynein - dynein 1b - driving the transport in the opposite direction. How these motor proteins cooperate and how their action is regulated is largely unknown. Here we apply high-sensitivity, quantitative wide-field fluorescence microscopy, which allows visualization of fluorescent motor proteins at endogenous expression levels. To this end, we have generated transgenic worms using Mos1-mediated single-copy integration of transgenes encoding fluorescently-labeled IFT-kinesins. We show that kinesins relay on the cilium structure to drive IFT: kinesin- II is the key player in the initial stage, while OSM-3 takes over further on. At the base of the tip, kinesin-II combines with tens of other kinesin-II's to form trains of motors. On the so-called middle segment of the cilium, kinesin-II motors progressively detach from the microtubules and are almost instantly transported back. At the same time, OSM-3 motors increasingly take over and transport cargo further, to the cilium tip. These findings shed new light on cooperativity of motor proteins driving intracellular transport in vivo.

3351-Pos Board B506
Huntington-Associated Phosphorylation of Kinesin-1 Enhances Autoinhibition in a Phosphomimic
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One of the consequences of the triplet expansion in Huntington's disease is inhibition of fast axonal transport (FAT). Phosphorylation of Ser176 in human kinesin-1 by JNK3 has been implicated in this inhibition (Morfini, et al., Nature Neruo. 12, 866 (2009)). To investigate the molecular basis for the inhibition of FAT, we have generated the S182E phosphomimic of the homologous residue in *Drosophila* kinesin-1. When introduced into short dimer of motor domains, the S182E mutation produces a 30% decrease in the maximum rate of microtubule-stimulated ATPase rate in solution and a similar reduction in the sliding rate of axonemes in a multimotor sliding assay. This only modest decrease suggests that direct inhibition of motility is not likely to be the principal cause of the pronounced inhibition of FAT. However, free kinesin is known to be autoinhibited through the binding of a tail domain to a dimer of motor domains (heads) and the Ser182 phosphorylation site is near the tail binding site on the heads (Kaan, et al., Science 333, 883 (2011)) where it could influence autoinhibition. One possibility is that the increased negative charge on the heads due to phosphorylation of Ser182 could produce a stronger interaction with the positively charge tail domain that would strengthen autoinhibition and inhibit FAT. To test the effect of the phosphomimic on autoinhibition, the binding of a monomeric tail domain to a dimer of motor domains was determined using a FRET assay. In 100 mM KCl, monomeric tail domains bind to mutant S182E heads three-fold more tightly than to wild type heads. This suggest that inhibition of FAT may principally be due to enhanced tail binding and accompanying autoinhibition of kinesin-1 following phosphorylation.
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3352-Pos Board B507
Biomimetic Cilia as a Model Ependymal Cilia System
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Cilia are ubiquitous throughout the human body and serve a variety of functions. Human lung cilia in particular have been widely studied due to the prevalence of ciliary diseases such as cystic fibrosis. Less-well-studied are ependymal cilia, which are responsible for transporting cerebrospinal fluid throughout the ventricular system; however, their response to increased viscous loading during infection may be critical in understanding the pathology and treatment of meningitis and other inflammatory diseases.
While ependymal cilia and human lung cilia are morphologically homologous, it has been shown in ex vivo studies that they respond very differently to increased viscous loading: lung cilia maintain a constant beat frequency but show decreased beat amplitude, while ependymal cilia maintain amplitude

but decrease frequency. This difference may have dramatic implications in the clearance of viscoelastic fluids. However, the physical mechanisms behind this clearance are not well understood.
We present therefore an artificial, biomimetic system which replicates the features of ependymal cilia as a tool for understanding the biological system. These biomimetic cilia are constructed of a material which is a composite of magnetic nanoparticles and silicone polymer. The composite has a high magnetic content (up to 50% wt.) and is homogenous at length scales below 100 nm, making it ideally suited to the fabrication of micro-scale magnetic actuators. The cilia are templated in a porous polycarbonate track-etched membrane which is subsequently dissolved with chloroform. The resulting cilia are 25 microns tall by 1 micron in diameter and may be actuated with an external magnetic field. A large array of cilia can be implemented in a microfluidic geometry for analysis of tracer particle movement to elucidate the interaction of cilia with a viscoelastic fluid.

3353-Pos Board B508
Probing the Role of Rotational Dynamics in Cellular Transport
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While it can generally be said that cellular function is critically dependent on the fidelity of cargo transport, processive transport is even more important in the axons and dendrites of neurons, where a cell must regulate populations of molecules on length scales that can range up to meters. Consequently, much effort has been made to investigate the translocation of cargoes in neurons and the properties of the motors responsible therein. Though biocompatible fluorophores have become increasingly powerful tools for study of motor-driven transport, they suffer from photobleaching and require bright illumination which can be toxic to live cells. Most conventional fluorescent approaches are further limited by the lack of oreintation information they provide. On the other hand, with the small diameter of neurites and the high levels of traffic they support through a crowded environment, orientation of the cargoes relative to the cytoskeletal tracks they are moving on can be vital. We present an experimental approach making use of dark field optical microscopy and gold nanorods as reporters of both lateral translocation as well as orientation of cargo in neurons. using relatively low illumination intensity, we can measure dynamics of single cargoes moving in the image plane and resolve changes in the azimuth and polar angles all at millisecond time scales. Furthermore, the gold nanorods can be specifically delivered to the cell body or axon terminal by culturing the neurons in microfluidic devices with separate chambers, enabling the investigator to resolve differences between retrograde and anterograde transport. The ability to track axonal transport with a high temporal and spatial dynamic range reveals several kinds of orientational changes of moving cargoes that correlate with transport dynamics, allowing more detailed inferences into changes in the activity of molecular motors.

Photosynthesis

3354-Pos Board B509
Donor Side-Induced Photoinhibition in Photosystem II
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To investigate the photoinhibition sites in PSII at different pH (pH 5.5, 7.0 and 8.5) and at the action of heavy metals (Cd^{2+} and Co^{2+}) were monitored by delayed fluorescence of chlorophyll *a* in the millisecond range (ms-DF). During photoinhibition of PSII membranes by excess light (4000 μmol photons m^{-2} s^{-1}) the fast and steady-state phases of ms-DF induction curve were monitored. We have observed strong photoinhibition effect on phases of ms-DF at the elevated pH values. At acidic pH photoinhibition of the fast and steady-state phases were less pronounced and, in addition, protective role of an exogenous acceptor PpBQ has been observed. In view of our previous results we suggest that change in equilibrium between Y_Z [$(P_{680}Q_A^-) \leftrightarrow (Y_Z^{\bullet+})$] and Mn_4O_5Ca-cluster [$(P_{680}Q_A^-) \leftrightarrow (S_{i+1}^+)$] taking place when pH of medium is changed from low to high values. At high pH this leads to the donor side induced photoinhibition and the destruction of the Mn_4O_5Ca-cluster. Under simultaneously action of Cd^{2+} and Co^{2+} ions and photoinhibition PSII become very sensitive to action of inhibitory illumination against the background of heavy metals. This inhibitory effect appears very strongly in the case of inhibition of donor side of PSII by Cd^{2+}. The main targets of the metals action may be partners for recombination with P_{680}^+, depend on medium pH, either Yz or Mn_4O_5Ca-cluster on the donor site for Cd^{2+} and Co^{2+} ions and between Q_A and Q_B on the acceptor site for Co^{2+} ions.

3355-Pos Board B510
Two Dimensional Broadband Electronic Spectroscopy of Photosystem II Core Complexes
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Studying the energy transfer and conversion processes in natural photosystems has both fundamental importance and relevance to the development of artificial light harvesting devices. Of particular interest is the D1/D2-cyt b559 reaction center of photosystem II (D1D2 RC), where the primary charge separation events associated with the evolution of molecular oxygen in photosynthesis occur. using transient 2D electronic spectroscopy (2DES), the energy transfer and charge separation processes in this system can be studied with femtosecond temporal resolution. Previously we have reported 2DES studies of the D1D2 RC in the Qy region where the electronic transitions associated with charge separation are found. This region contains overlapping transitions from all of the constituent chlorophyll and pheophytin pigments in the D1D2 RC, complicating the interpretation of the spectroscopic dynamics. Here we present broadband 2DES data spanning 460 - 700 nm along the detection axis, enabling the use of other electronic transitions to facilitate understanding of the energy transfer and charge separation processes. We discuss preliminary 2DES results on highly functional core complexes from BBY particles, which include the D1D2 RC along with the CP43, CP47 and psbO subunits. By studying the more intact and functional core complex, we aim to determine whether more reduced systems such as D1D2 RC experience changes in excitonic couplings or fundamental charge separation as a result of purification.

3356-Pos Board B511
Selective Abolishment of Electron Transfer at A1 Site in Cyanobacterial Photosystem I with Minimal Structural Disturbance
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Photosystem I/PSI is an integral membrane protein complex that harvests solar energy into reducing power. PSI electron transfer cofactors consist of 6 chlorophylls, 2 phylloquinones (PhQs) and 3 Fe_4S_4 clusters. The electron transfer in PSI of the cyanobacterium *Synechocystis* sp. PCC 6803 has been studied in two site-directed variants, $M688H_{PsaA}$ and $M668H_{PsaB}$ variants. In those two variants, the Met residue that acts as axial ligand to the primary electron acceptor A_0 has been substituted with a His either on the PsaA($M688H_{PsaA}$) or the PsaB($M668H_{PsaB}$) subunits. Room temperature transient EPR study indicated that the Met to His mutation on the A-sided ($M688H_{PsaA}$) variant slowed down the forward electron transfer from A_{1A} to F_X. This might resulted from the formation of a second hydrogen bond to the C1 carbonyl oxygen of PhQ in the A_{1A} site. Flash-induced absorbance change at 480nm confirmed the slower forward electron transfer from A_{1A} to F_X in the $M688H_{PsaA}$ variant. Likewise, forward electron transfer from A_{1B} to F_X was also slowed down in the B-sided variant. This result supported the formation of a second hydrogen bond to the PhQ in the A_{1B} site as well. Furthermore, we detected new components of charge recombination kinetics in these two variants. Each of the components corresponded to charge recombination between A_{1A} to $P_{700}+$ in $M688H_{PsaA}$ PSI and A_{1B} to $P_{700}+$ in $M668H_{PsaB}$ PSI, respectively. We propose that the Met to His mutation abolished the forward electron transfer from A_{1A} to F_X in the $M688H_{PsaA}$ variant and A_{1B} to F_X in the $M668H_{PsaB}$ variant. This is consistent with the establishment of a second hydrogen bond to the PhQ. Two models of the primary charge separation in PSI are discussed in the context of this finding.

3357-Pos Board B512
Theoretical Study of Electron Transfer Rate from Phylloquinones to Iron-Sulfur Cluster (F_x) in Photosystem I
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Photosystem I (PSI) is one of the two multi-subunit, pigment-protein complexes that drive the initial processes of light utilization in oxygenic photosynthesis. The PSI complex has approximately 100 packed chlorophyll (Chl) molecules that form the antenna and functional redox cofactors comprising the reaction center (RC). The latter cofactors are arranged in two pseudo-symmetric branches, starting from P700, and then split into two branches with the ec2 and ec3 chlorophylls and phylloquinone (A_1) on each branch, and then rejoin at the iron-sulfur cluster F_X. All of these are bound by the heterodimer of the PsaA and PsaB subunits, while the terminal F_A and F_B clusters are bound by PsaC. Electron transfer (ET) along the two branches has been studied by various investigators, and it was established that both branches are active in electron transfer, but that electron transfer to F_X was ~10-fold faster from PhQ_B than from PhQ_A.